Amendments to the Specification

At the indicated page and line numbers, please replace the existing sections or paragraphs, as the case may be, with the ones set forth below.

(Page 1, lines 1 and 2) ~~POLYKETIDES, THEIR PREPARATION, AND MATERIALS FOR USE THEREIN~~ NUCLEIC ACID MOLECULES ENCODING MODIFIED POLYKETIDE SYNTHASES CONTAINING POLYLINKERS AND USES THEREOF

(Page 7, lines 23 through 28) Desirably the nucleic acids of the invention encode a loading module and/or one or more extension modules. More detail concerning varieties of loading modules may be found in our copending international patent application (PCT/GB99/02044), entitled "Polyketides and their synthesis", filed 29 June 1999.

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(Page 8, line 17 through page 9, line 2) The genes encoding numerous examples of Type I PKSs have been sequenced and these sequences disclosed in publicly available DNA and protein sequence databases including Genbank, EMBL, and Swissprot. For example the sequences are available for the PKSs governing the synthesis of, respectively, erythromycin (Cortes, J. et al. Nature (1990) 348: 176-178; accession number X62569, Donadio, S. et al. Science (1991) 252: 675-679; accession number M63677); rapamycin (Schwecke, T. et al. Proc. Natl. Acad. Sci. USA (1995) 92: 7839-7843; accession number X86780); rifamycin (August et al. (1998); accession number AF040570); and tylosin (Eli Lily, accession number U78289), among others. Furthermore, figure 7 (SEQ ID NO: 1) herein shows the nucleic acid sequence encoding the first two modules of the avermectin PKS from S. avermitilis; this may be used as an alternative source for the inserts used in certain of the examples.

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(Page 10, line 26 through page 11, line 5) These new restriction sites are situated partly in DNA encoding a linker region near positions where the polyketide synthase is hydrolysed by proteolytic enzymes (vide supra). While some of the restriction sites lie in DNA encoding regions of low homology, others are situated in DNA encoding highly conserved regions (Figure 1 showing SEQ ID NOs: 36, 38, 40, 42, 44, 46, 48, 50, 52, and 54 in the left column from top to bottom and SEQ ID NOs: 37, 39, 41, 43, 45, 47, 49, 51, 53, and 55 in the right column from top to bottom). The introduction of recognition sites for the enzymes AvrII, BglII, Bsu36I and NheI does not change the amino acid sequence in DEBS module 2. In the other five cases (SnaBI, PstI, SpeI, Nsi, HpaI) the amino acid sequence is changed (Figure 2 showing SEQ ID NOs: 36 (left) and 37 (right) and the changes for SnaBI, SEQ ID NO: 56; PstI, SEQ ID NO: 57; SpeI, SEQ ID NO: 58; Nsi, SEQ ID NO: 59; and NheI, SEQ ID NO: 60). These changes do not affect the activity of the protein (see example 6).

B3

(Page 14, lines 7 through 20) The approximately 1.47 kbp DNA fragment of the eryAI gene of S. erythraea was amplified by PCR using as primers the synthetic oligonucleotides: 5'-TACCTAGGCCGGGCCGGACTGGTCGACCTGCCGGGTT-3' (SEQ ID NO:2) and 5'-ATGTTAACCGGTCGCGCAGGCTCTCCGTCT-3' (SEQ ID NO: 3) and plasmid pNTEP2 (Oliynyk, M. et al., Chemistry and Biology (1996) 3: 833-839; W098/01546) as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK02 was identified by its restriction pattern and DNA sequencing.

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(Page 14, line 24 through page 15, line 3) The approximately 1.12 kbp DNA fragment of the eryAI gene of S. erythraea was amplified by PCR using as primers the synthetic oligonucleotides: 5'-ATGTTAACGGGTCTGCCGCGTGCCGAGCGGAC-3' (SEQ ID NO: 4) and 5'-CTTCTAGACTATGAATTCCCTCCGCCCAGC-3' (SEQ ID NO: 5) and plasmid pNTEPH as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK03 was identified by its restriction pattern and DNA sequencing.

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(Page 16, lines 2 through 11) The two synthetic oligonucleotides P1f and P1b (Figure 4 showing oligonucleotides P1f (SEQ ID NO: 61) and P1b (SEQ ID NO: 62) and the oligonucleotides annealed (SEQ ID NOs: 63-66 from top to bottom)) were each dissolved in TE-buffer. 10 μL of each solution (0.5nmol/μL) were mixed and heated for 2 minutes at 65C and then slowly cooled down to room temperature. Plasmid pJLK07 was digested with AvrII and HpaI and ligated with the annealed oligonucleotides. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK114 was identified by its restriction pattern.

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(Page 20, lines 2 through 15) The approximately 1.4 kbp DNA fragment of the eryAI gene of S. erythraea encoding the reductive loop of module 2 was amplified by PCR using as primers the synthetic oligonucleotides: 5'-ATACTAGTCCTCGTGACGAGCTCGACGG-3' (SEQ ID NO: 6) and 5'-TAATGCATCCGGTTCTCCGGCCCGCTCGCT-3' (SEQ ID NO: 7) and pNTEP2 as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18,

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which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK118 was identified by its restriction pattern and DNA sequencing.

B7

(Page 22, lines 13 through 27) The approximately 3.2 kbp DNA segment of the rapC gene of S. hygroscopicus encoding the reductive loop of module 13 was amplified by PCR using as primers the synthetic oligonucleotides:
5'-TAAGATCTTCCGACCTACGCCTTCCAAC-3' (SEQ ID NO: 8) and
5'-TAATGCATCGACCTCGTTGCGTGCCGCGGT-3' (SEQ ID NO: 9) and cosmid cos 31 (Schwecke, T. et al. (1995) Proc. Natl. Acad. Sci. USA 92: 7839-7843) as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK120 was identified by its restriction pattern and DNA sequencing.

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(Page 24, lines 14 through 28) The approximately 3.2 kbp DNA segment of the eryAII gene of S. erythraea encoding the reductive loop of module 4 was amplified by PCR using as primers the synthetic oligonucleotides:
5'-ATAGATCTGCCTACGTACCCGTTCGAACACCAGCGCTTC-3' (SEQ ID NO: 10)
and 5'-ATCCTCAGGTTCGGCCCTGCCGCCTCGGCCTGCCCGGCGGCGCGCAGCTT-3'
(SEQ ID NO: 11) and cosmid cos4B (cosmid containing the erythromycin PKS) as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and

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B9 individual colonies were checked for their plasmid content. The desired plasmid pJLK32.3 was identified by its restriction pattern and DNA sequencing.

(Page 26, line 24 through page 27, line 6) The approximately 2.2 kbp DNA segment of the rapB gene of S. hygroscopicus encoding the reductive loop of module 10 was amplified by PCR using as primers the synthetic oligonucleotides: 5'-TAAGATCTTCCGACGTACGCGTTCCAGC-3' (SEQ ID NO: 12) and 5'-ATGCTAGCCACTGCGCCGACGAATCACCGGTGG-3' (SEQ ID NO: 13) and as template an approximately 7 kbp fragment, which has been obtained by digestion of cosmid cos 26 (Schwecke, T. et al. (1995) Proc. Natl. Acad. Sci. USA 92: 7839-7843) with B10 ScaI and SphI. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK121.1 was identified by its restriction pattern and DNA sequencing.

(Page 28, line 30 through page 29, line 11) The approximately 1.6 kbp DNA segment of the tylosin PKS gene of S. fradiae encoding the reductive loop of module 1 was amplified by PCR using as primers the synthetic oligonucleotides: 5'-TAAGATCTCCCTACGTACCCCTTCAACCAC-3' (SEQ ID NO: 14) and 5'-GCTAGCCGCCGCGCCAGCTCGGGC-3' (SEQ ID NO: 15) and B11 cosmid 6T (cosmid containing the tylosin-producing PKS genes) as template The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired

B11 plasmid pJLK33.1 was identified by its restriction pattern and DNA sequencing.

(Page 30, line 31 through page 31, line 12) The approximately 2.1 kbp DNA segment of the rifamycin PKS gene of Amycolatopsis mediterranei encoding the reductive loop of module 7 was amplified by PCR using as primers the synthetic oligonucleotides: 5'-CCTACGTACGCCTTCGACCACCAGCACTT-3' (SEQ ID NO: 16) and 5'-CGGCTAGCGGGCGTTCCAGGCCGCCGTCCT (SEQ ID NO: 17) and cosmid 6 (cosmid starting at 35727 and going beyond 76199, numbers according to accession number AF040570) as template. The PCR product was treated with T4 polynucleotide kinase and B12 then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pUCRIF7 was identified by its restriction pattern and DNA sequencing.

(Page 33, lines 7 through 21) The approximately 6.1 kbp DNA segment of the erythromycin PKS gene cluster of S. erythraea encoding the DNA fragment from the beginning of the ACP of module 2 to the beginning of the ACP of module 3 was amplified by PCR using as primers the synthetic oligonucleotides: 5'-TACCTGAGGGACCGGCTAGCGGGTCTGCCGCGTG-3' (SEQ ID NO: 18) and 5'-ATGCTAGCCGTTGTGCCGGCTCGCCGGTCGGTCC-3' B13 (SEQ ID NO: 19) and plasmid pBAM25 as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK50 was identified by its restriction pattern and DNA

sequencing.

(Page 39, line 26 through page 40, line 8) The approximately 2.4 kbp DNA segment of the avermectin PKS gene of S. avermitilis encoding the region upstream of the reductive loop of module 2 was amplified by PCR using as primers the synthetic oligonucleotides:
5'-GACGCCGAATTCTTCGGCATCAGCCCCCGCGAAG-3' (SEQ ID NO: 20) and
5'-GAGCTAGCAGGTGGGGAGATCTAGGTGGGTGTGGGTGTGGGGTTGGTTGTGGTGGTGG GTGTA-3' (SEQ ID NO: 21) and plasmid pIG22 (Galloway, I. S. (1998) Thesis, University of Cambridge, UK) as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK130 was identified by its restriction pattern and DNA sequencing.

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(Page 40, lines 12 through 26) The approximately 2.0 kbp DNA segment of the avermectin PKS gene of S. avermitilis encoding the region downstream of the reductive loop of module 2 was amplified by PCR using as primers the synthetic oligonucleotides:
5'-GCCCGGCTAGCCGGCCAGACACACGAACAACAGC-3' (SEQ ID NO: 22) and
5'-GGGAATTCCTCGAGGATGACGTGGGCGTTGGTGC-3' (SEQ ID NO: 23) and plasmid pIG25 (Galloway, I. S. (1998) Thesis, University of Cambridge, UK) as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK131 was identified by its restriction

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B15 Cont pattern and DNA sequencing.

(Page 41, lines 16 through 30) The approximately 1.9 kbp DNA segment of the erythromycin gene cluster of S. erythraea encoding the erythromycin resistance was amplified by PCR using as primers the synthetic oligonucleotides: 5'-TAAGATCTAGCGCTCCGAGGTTCTTGCCCG-3' (SEQ ID NO: 24) and 5'-ATGCTAGCCTACCGCTGCCCGGGTCCGCCG-3' (SEQ IS NO: 25) and plasmid pRH3 (Dhillon, N, et al. Molecular Microbiology (1989) 3: 1405-1414) as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK134 was identified by its restriction pattern and DNA sequencing.

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(Page 46, line 25 through page 47, line 8) The approximately 1.7 kbp DNA segment of the gene of the avermectin PKS of S. avermitilis encoding the reductive loop of module 1 was amplified by PCR using the following synthetic oligonucleotides as primers: 5'-CCTAGATCCGCCCACCTACCCCTTCCAACACCAG-3' (SEQ ID NO: 26) and 5'-TGGGCTAGCGTTTTGTGCAACTCCGCCGGTGGAGTG-3' (SEQ ID NO: 27) and as template either plasmid pIG155, which contains the first two modules of the avermectin PKS cloned into plasmid pT7-7, or chromosomal DNA of Streptomyces avermitilis. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompotent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pIG67 was identified by its restriction pattern and by DNA

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sequencing.

(Page 48, line 24 through page 49, line 8) The approximately 1.7 kbp DNA segment of the gene of the avermectin PKS of S. avermitilis encoding the reductive loop of module 1 was amplified by PCR using the following synthetic oligonucleotides as primers:
5'-TGGCTGCAGAGCTCACAGCCGGGTGCCGGATCCGGTT-3' (SEQ ID NO: 28)
and 5'-TTTCCTCAGGTCCGCCGGTGGAGTGGGGCGCTGGAC-3' (SEQ ID NO: 29)
and as template either plasmid pIG155, which contains the first two modules of the avermectin PKS cloned into plasmid pT7-7, or chromosomal DNA of Streptomyces avermitilis. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompotent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pIG68 was identified by its restriction pattern and by DNA sequencing.

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(Page 51, lines 11 through 27) The approximately 2.4 kbp DNA segment of the gene of the avermectin PKS of S. avermitilis encoding the reductive loop of module 2 was amplified by PCR using the following synthetic oligonucleotides as primers:
5'-CCTAGATCTCCCCACCTACCCCTTCCAACACCACCACTACTG-3' (SEQ ID NO: 30) and 5'-CCGGCTAGCCGGGCGTGCAGCTGGGCGCCGTTGTCCGCAC-3' (SEQ ID NO: 31) and as template either plasmid pIG155, which contains the first two modules of the avermectin PKS cloned into plasmid pT7-7, or chromosomal DNA of Streptomyces avermitilis. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform

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electrocompotent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pIG69 was identified by its restriction pattern and by DNA sequencing.

B19 cont

(Page 53, line 14 through page 54, line 5) The approximately 2.4 kbp DNA segment of the gene of the avermectin PKS of S. avermitilis encoding the reductive loop of module 2 was amplified by PCR using the following synthetic oligonucleotides as primers: 5'-CCCTACGTACCCCTTCCAACACCACTACTGGCTCGAAAG-3' (SEQ ID NO: 32) and 5'-GGCCCTCAGGTGGGCGCCGTTGTCCGCACCACCGGTA-3' (SEQ ID NO: 33) as template either plasmid pIG155, which contains the first two modules of the avermectin PKS cloned into plasmid pT7-7, or chromosomal DNA of Streptomyces avermitilis. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompotent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pIG70 was identified by its restriction pattern and by DNA sequencing.

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(Page 55, line 25 through page 56, line 7) The approximately 3.2 kbp DNA segment of the rapC gene of S. hygroscopicus encoding the reductive loop of module 13 was amplified by PCR using as primers the synthetic oligonucleotides: 5'-TACCTAGGCACCACCACAACCCGGGTA-3' (SEQ ID NO: 34) and 5'-TACAATTGGCCCGCGAGTCCCCGACGCT-3' (SEQ ID NO: 35) and cosmid cos 31 (Schwecke, T. et al. (1995) Proc. Natl. Acad. Sci. USA 92: 7839-7843) as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with SmaI and then treated with alkaline phosphatase. The ligation

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mixture was used to transform electrocompetent E. coli DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK120a was identified by its restriction pattern and DNA sequencing.